NEMATODES AND ASSOCIATED FUNGI FOUND IN FOREST NURSERIES

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INTRODUCTION

A few references were found regarding the activities of pathogenic fungi in forest nurseries in Portugal. Comparatively little information is available on the importance of plant-parasitic nematodes as components in seedling disease complexes. Having frequently observed the typical symptoms of damping-off of seedlings in forest nurseries, a study was undertaken in order to determine whether nematodes are associated with the disease and, if so, to ascertain their genera and their numbers in the soil adhering to seedling roots. In addition laboratory tests were made to investigate the rôle of nematodes in the damping-off disease on forest seedlings.

MATERIALS AND METHODS

Soil samples were collected from forest nurseries (Table 1) at Amarante (Province of Douro Litoral), Boticas and Pedras Salgadas (Province of Trás-os-Montes e Alto Douro) in the North of Portugal.

The samples were obtained by removing the adhering soil to the roots of seedlings showing symptoms of damping-off. The nematodes were extracted by using a modified Baermann funnel technique. With the aid of a stereomicroscope the nematodes were counted and then concentrated for identification; this was done by using a compound microscope. At the same time, the fungi were isolated from the damped-off seedlings.

On the other hand we tried to inoculate seedlings of *Pinus pinaster* Aiton with *Fusarium oxysporum* Schlecht, associating a number of the test plants with specimens of *Xiphinema amarantum* Macara 1971 (¹). Three-month-old maritime pine seedlings — in all 108 in number — were grown in sterilized soil (200°C for 45m) in the greenhouse. Each of them was then transplanted to 14 cm diam. clay pot containing sterilized nursery soil (coarse sand, 34.05%; fine sand, 38.95%; silt, 10.36%; and clay, 16.26%); the pH of the soil ranged from 6.5 to 7.

The seedlings were treated one week after potting, according to the following criteria:

(¹) By the time the present paper was ready for press the author noticed that the species *X. amarantum* (Macara, 1970) had already been described under *X. sahelense* Dalmasso 1969 (Dalmasso, 1969), thus being the former a junior synonim of the latter.

A — Three seedlings were inoculated by a nematodes — *Fusarium* sp. combination (on account of a shortage of inoculum we had to use only 8, 4 and 2 nematodes respectivelly per pot).

B — Three seedlings were inoculated by nematodes (8, 4 and 2 nematodes, respectivelly, per pot).

C — Three seedlings were inoculated with *F. oxysporum*.

D — Three seedlings with their hypocotyl base and root crown injuried with entomological micropins were inoculated with *F. oxysporum* (according to a method described by Roth & Riker, 1943).

T — Three control seedlings.

The experiment had six replicates of the five treatments and the pots were arranged ramdomly in the greenhouse.

First of all the seedlings were inoculated by pouring an aqueous suspension of nematodes into a 5 cm hole made in the soil next to the seedling roots. Before inoculating, surface-sterilized nematodes were obtained at first by putting them in sterilized distilled water, then in a 0,05% aqueous solution of mercuric chloride for 15 sec, and at last in sterilized distilled water (Peacock, 1959). The fungus inoculum weighted 1,6 g and was introduced in the soil on the opposite sides of the plant hypocotyl and root crown. A standard watering procedure was employed. Greenhouse temperatures were in quasi-field condition ~~ones~~ and during three months ranged from 6 to 29°C, while soil temperature in the pots oscillated from 6 to 26°C. The experiment was concluded three months after the nematodes and fungi had been added. Afterwards the seedlings were removed from the pots and the soil was gently shaken from the roots. A suitable fragment was removed from the diseased hypocotyl and root crown of each damped-off seedling. It was sterilized for 3 minutes in 0,1% mercuric chloride, and washed twice in sterile water. Then the fungus was re-isolated on malt agar ($pH=6,8$). Sections 40 µ thick were cut from base of the hypocotyl and root crown and stained with cotton blue.

As an additional trial we poured into a sterile Petri dish some sterilized distilled water containing 8 nematodes for one maritime pine seedling, in order to observe the behaviour of the nematodes.

RESULTS AND DISCUSSION

Nematodes of the family Dorylaimidae de Man 1876 and in association with fungi of the genus *Fusarium* were detected in the rhizosphere of forest seedlings. The results of this study are presented in Table 1. Only two plant-parasitic nematodes were found: *Paralongidorus* sp. in one of the nurseries (at Pedras Salgadas) in a low percentage (0,5%), and *X. amarantum* which had the most widespread occurrence, especially in the rhizosphere of *P. laricio* Poir (20%), was found in all the three nurseries. However the most prevalent group of nematodes, also found in all three nurseries, was the genus *Eudorylaimus* (possibly more than one species) which is known to prey on other nematodes and other members of the soil microfauna. Some of these nematodes may also feed on soil-borne fungi. At Boticas we also detected empty cysts of *Heterodera rostochiensis* Woll. (Fam. Heteroderidae). The information in Table 1 does not necessarily represent true relationships. It represents each a mean~~s~~, number per 100 ml of soil, only for the parasitic nematodes. Moreover the seedlings selected for this study were those showing some symptoms of damping-off.

Table 1 — *Nematodes related with fungi of the genus* Fusarium *in the rhizosphere of forest seedlings.*

	Nurseries									
	Betula alba		*P. pinaster*	*P. sylvestris*			*P. laricio*		*Fagus sylvatica*	*Juglans regia*
	Amarante	Boticas	Amarante	Amarante	Boticas	Pedras Salgadas	Boticas	Pedras Salgadas	Pedras Salgadas	Pedras Salgadas
Nematodes:										
Eudorylaimus sp.	+	+	+	+	+	+	+	+	+	+
Paralongidorus sp. (Mean number per 100 ml of soil)	—	—	—	—	—	—	—	—	+ (0,5)	—
X. amarantum	+	+	+	+	+	+	+	+	—	+
(Mean number per 100 ml of soil)	(11)	(7)	(8)	(10)	(9)	(5)	(20)	(4)	—	(5)
Fungi:										
Fusarium sp.	+	+	—	+	+	+	+	+	+	+
F. oxysporum	—	—	+	—	—	—	—	—	—	—
Phytophthora sp.	—	—	—	—	—	—	—	—	—	+

The results of the studies on experimental pathogenicity are presented in Table 2. The criterion used to decide whether a fungus was able to attack the host or not was the visible evidence of a constriction in the hypocotyl and root crown, where the cortical tissue was destroyed (Fig. 1), causing the colapse of the seedling, a typical damping-off symptom. The histopathological examination showed hyphae growing from the cortex. The nematodes never penetrated into the roots. On examining the sections there was no visual evidence of the wounds made by the nematodes during feeding. The disease symptoms that appeared along the needles were not used as a sign of infection, since they were of common occurrence, even on control seedlings, owing to unknown causes, inclusive nutritional ones. The typical symptoms of damping-off were detected in larger number on seedlings under treatment A. The induced damping-off of the seedlings under treatments A, C and D appeared at the same time, i.e., forty six days after inoculation. The seedlings with damping-off were then removed from the pots for confirmation and their numbers were recorded. The control seedlings were, as expected, in a most advanced stage of development (Figs. 1 and 2). Results

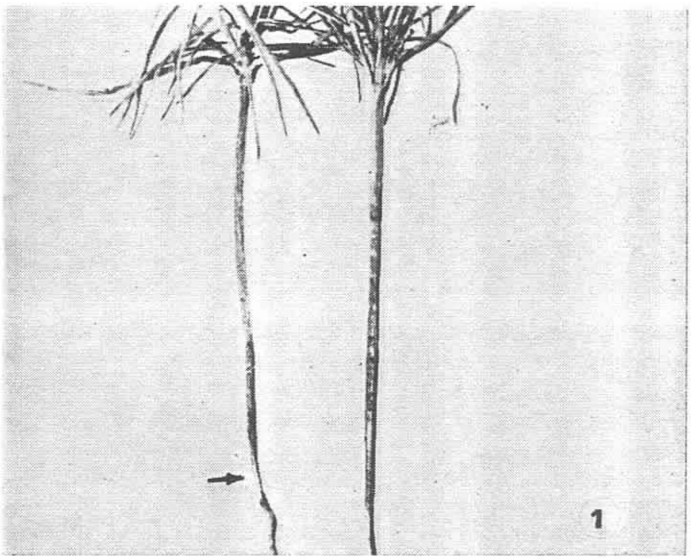

Fig. 1 — *Diseased hypocotyl and root crown (left) and control (right). The arrow indicates the constriction.*

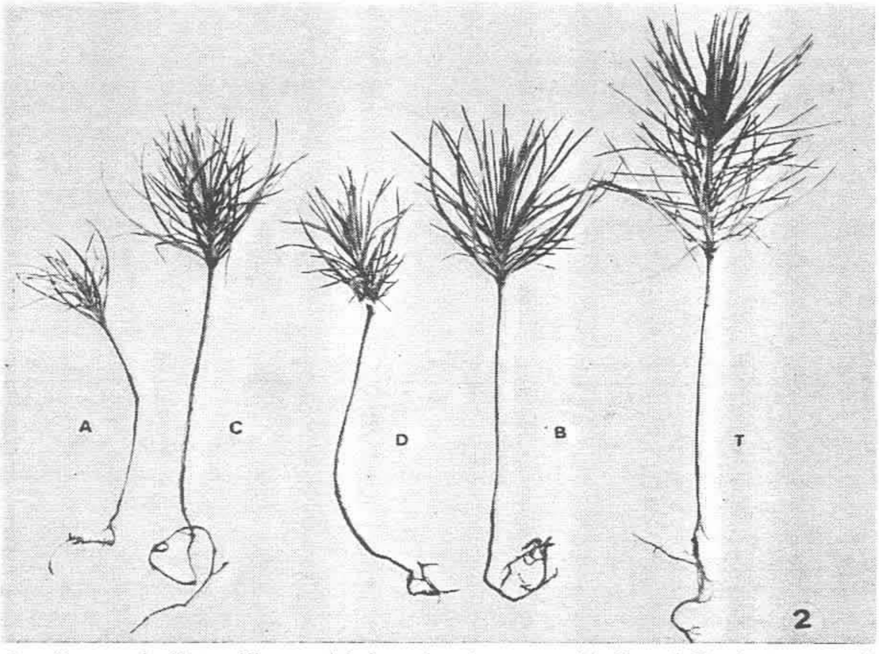

Fig. 2 — *Damped-off seedlings with less development (A, C and D) than control (T). Seedling (B) almost with the same appearance as control.*

from the different replications were consistent and indicate that nematodes (treatment A) and mechanical injuries to the base of hypocotyl and root crown (treatment D) greatly increased the incidence of damping-off in the presence of the fungus. The difference between the results of treatments A and D was not statistically significant (P=0,5), if compared with each other, but in relation to treatment C they were statistically significant (P=0,05). The additional trial (referred too in Material and Methods), carried out in a Petri dish, showed that the feeding activity of some nematodes took place on the root, hypocotyl and root crown. The feeding began within one hour after the nematodes were placed near the root. The feeding wounds were made by an ectoparasitic process of puncturing the cells with the stylet. Thus, it may be concluded that at the base of the hypocotyl and root crown the nematode *X. amarantum* increases the incidence of damping-off by causing wounds on the hypocotyl and root crown, through which damping-off fungi might enter and at the same time thus predisposing the seedlings to their attacks. Treatment B, when compared to the control, was not statistically significant (P=0,5). There were no appreciable differences in the results regarding the respective numbers of nematodes: 8, 4 and 2 in treatments A and B (Table 2). The nematodes observed did not seem to multiply on these roots. Many problems remain yet to be explained which can only be clarified through future research.

Table 2 — *Results of the experimental studies on the pathogenicity.*

Presence or absence of disease	Type of treatment								
	A. Number of seedlings with *Fusarium* + nematodes 18			B. Number of seedlings with nematodes 18			C. Number of seedlings with *Fusarium* 18	D. Number of pierced seedlings 18	T. Number of control seedlings 18
	6 With 8 nematodes per seedling	6 With 4 nematodes per seedling	6 With 2 nematodes per seedling	6 With 8 nematodes per seedling	6 With 4 nematodes per seedling	6 With 2 nematodes per seedling			
With damping-off (%)	5	5	4	—	—	—	7 (38,8)	13 (72,2)	—
Without damping-off (%)	1	1	2	—	—	—	11 (61,1)	5 (27,7)	—
Without damping-off but with some discoloured or dead needles (%)	1	—	2	2	3	4	7 (38,8)	3 (16,6)	7 (38,8)
With damping-off but without discoloured or dead needles (%)	2	1	2	—	—	—	5 (27,7)	8 (44,4)	—

RESUMO

NEMÁTODOS E FUNGOS ASSOCIADOS OBSERVADOS EM VIVEIROS FLORESTAIS

Em amostras de terra de viveiros florestais (Tab. 1) foram detectados nemátodos da família Dorylaimidae de Man, 1876, em associação com fungos do género *Fusarium* na rizosfera de plântulas das seguintes espécies florestais: *Betula alba* L., *Fagus sylvatica* L., *Pinus laricio* Poir., *P. pinaster* Aiton e *P. sylvestris* L. Em situação semelhante observou-se um fungo do género *Phytophthora* em associação com *Juglans regia* L.

Pela primeira vez em Portugal investigou-se a acção dos nemátodos na doença «emurchecimento» (Damping-off) em plântulas florestais, isto é, por causarem feridas nas raízes, através das quais podem penetrar os fungos agentes causais dessa doença; os nemátodos podem enfraquecer as plântulas assim predispostas aos ataques dos fungos.

Ensaiaram-se plântulas de *P. pinaster* inoculadas com *Fusarium* associado com espécimes de *Xiphinema amarantum* Macara 1971. As plântulas desenvolveram-se na estufa em condições quase naturais. O nemátodo e o fungo foram detectados em viveiros florestais das províncias do Douro Litoral, Trás-os-Montes e Alto Douro.

Comparou-se a sintomatologia e histopatologia de várias plântulas doentes e sãs de 3 meses de idade seleccionadas naqueles viveiros.

Concluiu-se que na base do hipocótilo e no colo radicular o nemátodo pode aumentar a incidência do «emurchecimento» causado por *Fusarium* sp. no pinheiro marítimo.

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